AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

MYCOLOGY

A Short-Tandem-Repeat Assay (*Mmy*STR) for Studying Genetic Variation in *Madurella mycetomatis*

Bertrand Nyuykonge,[a] Kimberly Eadie,[a] Willemien H. A. Zandijk,[a] Sarah A. Ahmed,[b,c] Marie Desnos-Ollivier,[d] Ahmed H. Fahal,[e] Sybren de Hoog,[b] Annelies Verbon,[a] Wendy W. J. van de Sande,[a] Corné H. W. Klaassen[a]

[a]Erasmus MC University Medical Center Rotterdam, Department of Medical Microbiology and Infectious Diseases, Rotterdam, The Netherlands
[b]Center of Expertise in Mycology of Radboud University Medical Center/Canisius Wilhelmina Hospital, Nijmegen, The Netherlands
[c]Faculty of Medical Laboratory Sciences, University of Khartoum, Khartoum, Sudan
[d]Institut Pasteur, CNRS UMR 2000, National Reference Center for Invasive Mycoses & Antifungals, Molecular Mycology Unit, Institut Pasteur, Paris, France
[e]Mycetoma Research Center, Khartoum, Sudan

ABSTRACT *Madurella mycetomatis* is the major causative agent of eumycetoma, a neglected tropical infection characterized by painless subcutaneous lesions, inflammation, and grains draining from multiple sinuses. To study the epidemiology of mycetoma, a robust discriminatory typing technique is needed. We describe the use of a short-tandem-repeat assay (*Mmy*STR) for genotyping of *M. mycetomatis* isolates predominantly from Sudan. Eleven microsatellite markers (3 dinucleotides, 4 trinucleotide repeats, and 4 tetranucleotide repeats) were selected from the *M. mycetomatis* MM55 genome using the Tandem Repeats Finder software. PCR amplification primers were designed for each microsatellite marker using primer3 software and amplified in a multicolor multiplex PCR approach. To establish the extent of genetic variation within the population, a collection of 120 clinical isolates from different regions was genotyped with this assay. The 11 selected *Mmy*STR markers showed a large genotypic heterogeneity. From a collection of 120 isolates, 108 different genotypes were obtained. Simpson's diversity index (D) value for individual markers ranged from 0.081 to 0.881, and the combined panel displayed an overall D value of 0.997. The *Mmy*STR assay demonstrated high stability, reproducibility, and specificity. The *Mmy*STR assay is a promising new typing technique that can be used to genotype isolates of *M. mycetomatis*. Apart from the possible contribution of host factors, the genetic diversity observed among this group of isolates might contribute to the different clinical manifestations of mycetoma. We recommend that the *Mmy*STR assay be used to establish a global reference database for future study of *M. mycetomatis* isolates.

KEYWORDS mycetoma, *Madurella mycetomatis*, genotyping, short tandem repeats, microsatellites, *Mmy*STR

Eumycetoma is a chronic granulomatous subcutaneous, infectious, and inflammatory neglected tropical disease caused by a wide range of filamentous fungi. It is characterized by large painless tumor-like masses in (sub)cutaneous tissue, with multiple draining sinuses. From these sinuses, grains are discharged, the color of which depends on the etiology of the causative agent (1–3). Eumycetoma is reported mostly in tropical and subtropical countries. However, imported cases have been reported in other parts of the world (4–6). Although more than 50 aetiologic agents have been reported to cause eumycetoma, the fungus *Madurella mycetomatis* is the dominant one, causing more than 75% of eumycetoma cases (2, 7–9). Currently, the environmental niche and the mechanism of transmission are not well understood; however, it has been suggested that mycetoma results from direct inoculation of the causative agent into the subcutaneous tissue from a thorn prick or contaminated soil (10). This is

Citation Nyuykonge B, Eadie K, Zandijk WHA, Ahmed SA, Desnos-Ollivier M, Fahal AH, de Hoog S, Verbon A, van de Sande WWJ, Klaassen CHW. 2021. A short-tandem-repeat assay (*Mmy*STR) for studying genetic variation in *Madurella mycetomatis*. J Clin Microbiol 59:e02331-20. https://doi.org/10.1128/JCM.02331-20.

Editor Kimberly E. Hanson, University of Utah

Address correspondence to Corné H. W. Klaassen, c.h.w.klaassen@erasmusmc.nl.

Received 9 September 2020
Returned for modification 17 October 2020
Accepted 1 December 2020
Accepted manuscript posted online 9 December 2020
Published 18 February 2021

probably also why the most commonly affected areas are the feet and hands, which account for more than 80% of all cases (11). In 2016, mycetoma was recognized as a neglected tropical disease (NTD) by WHO. Subsequently, considerable efforts have been made to map the burden of mycetoma to gain more knowledge about the distribution as well as the transmission of the disease (1). In these mapping efforts, genotyping will help to establish the genetic diversity and may provide some clues to the natural niche of *M. mycetomatis* and its epidemiology (12–16). These efforts could lead to the identification of preventive measures that may help reduce the burden of mycetoma. However, the current typing methods (12–16) have poor interlaboratory reproducibility, hampering exchange of data (8, 14, 17). In view of efforts toward global mapping of the burden of mycetoma, a typing technique that facilitates data exchange is mandatory.

Microsatellite assays, or short-tandem-repeat (STR) assays, have been used for studying the genetic diversity of several fungal species (18–23). Short-tandem-repeat assays have a wide range of advantages over previously used pattern-based methods due to their high reproducibility, their high discriminatory power, and the ease of communicating results from one lab to another (24). An STR assay is based on the amplification of DNA motifs of 2 to 10 bp, which are abundant in the genomes of most eukaryotes. This is followed by size determination using capillary electrophoresis, and the number of tandem repeats is then extrapolated from the size of the fragment (25). These motifs can be amplified in a multiplex format, permitting high-throughput analysis (18). An STR assay for mycetoma may be instrumental for the global mycetoma community in their surveillance programs and help to identify new foci of mycetoma. In the present study, we describe a multicolor multiplex panel of 11 STRs for studying genetic variability among *M. mycetomatis* isolates (*Mmy*STR assay). To demonstrate the superiority of this assay, we compared the *Mmy*STR assay data to genotyping data that were previously generated using amplified fragment length polymorphism (AFLP) and variable number tandem repeat (VNTR) assays.

MATERIALS AND METHODS

Isolates and DNA isolation. A total of 120 clinical isolates of *M. mycetomatis* originating from Sudan (100 isolates) and other regions (20 isolates) were included in the study. Nineteen of these were from India (4 isolates), Senegal (3 isolates), Mali (2 isolates), the United States (1 isolate), Canada (1 isolate), Netherlands (1 isolate), Chad (1 isolate), Peru (1 isolate), Algeria (1 isolate), Somalia (1 isolate), Niger (1 isolate), Morocco (1 isolate), and Switzerland (1 isolate), and 1 was from an unknown origin. The isolates from Netherlands, the United States, Canada, and Switzerland were considered to represent imported cases, as they originated from outside the so-called mycetoma belt (2). The isolates were from different patients, at different time points, and different geographical subregions. Fungal isolates or previously isolated DNA was obtained from various fungal collections, in particular, the Erasmus MC University Medical Center (Rotterdam, The Netherlands), the Westerdijk Fungal Biodiversity Institute (Utrecht, The Netherlands), UMIP (Institut Pasteur Collection, Paris, France) and the Mycetoma Research Centre (Khartoum, Sudan). Fungal DNA was isolated using the Zymo DNA extraction kit, as previously described (16). For specificity testing, several non-*M. mycetomatis* isolates were included: *Madurella tropicana* strain CBS 219.92, *Madurella fahalii* strain CBS 102793, *Madurella pseudomycetomatis* strain Mex2a, *Falciformispora senegalensis* strain CBS 132272, *Falciformispora tompkinsii* strain Na-5B, and *Medicopsis romeroi* strain CBS 135987. All strains used in the study were identified by sequencing their internal transcribed spacers (ITS); background information is provided in Data Set S1 in the supplemental material.

Identification of STR loci. The Tandem Repeats Finder software (26) was used to select candidate STR markers from the *M. mycetomatis* reference genome MM55 (CBS 108901; GenBank accession number LCTW02000000) (27). After an initial evaluation based on previously described criteria (18), an 11-marker microsatellite panel consisting of 3 dinucleotide repeats, 4 trinucleotide repeats, and 4 tetranucleotide repeat markers was selected for further use.

Primer design, PCR, and genotyping. Specific PCR amplification primers for the selected markers were designed using Primer3 software version 4.1.0 (Table 1) and ordered from Eurogentec (Liège, Belgium) or Thermo Fisher Scientific (Renfrewshire, United Kingdom). Three sets (*Mmy*STR2, *Mmy*STR3, and *Mmy*STR4), each amplifying 3 or 4 markers, were amplified using a multicolor multiplex PCR approach. For each panel, one of the primers was fluorescently labeled with either a FAM (6-carboxyfluorescein), VIC (2′-chloro-7′-phenyl-1,4-dichloro-6-carboxy-fluorescein), NED (2′-chloro-5′-fluoro-7′,8′-benzo-1,4-dichloro-6-carboxyfluorescein), or PET (polyethylene terephthalate) label (Table 1). The 25-μl amplification reaction mixture consisted of 0.5 μM concentrations of each specific primer and approximately 1 ng of genomic DNA in 1$\times$ PCR master mix (Roche Diagnostics). The 35-cycle amplification

TABLE 1 Characteristics of the selected STR markers

Marker	Repeat unit	Primer sequence (5′–3′)		Allelic range	Location
		Labeled	Unlabeled		
*Mmy*STR 2A	TG	FAM-TCCTGTTGCCTGACTGACTG	TGAAACCCGAACTTTCCTTG	12–39	Intergenic
*Mmy*STR 2B	CA	VIC-CACTCACTCCACGTCTTCCA	GGACGTAGGTGGGCATTTT	7–20	Intergenic
*Mmy*STR 2C	GT	NED-TGATGAGCTTCTCATTTTGGAG	CCTGGAAGAGATTCTGGGTTC	15–21	Intergenic
*Mmy*STR 3A	TAG	FAM-AGATATGTCGTGATCGGTTCG	ATGTAGATCGGAGCGGAAGA	8–33	Intergenic
*Mmy*STR 3B	CGT	VIC-TATCGATGTGGATCCGAGGT	TGGAGGAGCTGAAAGAATGG	5–30	Hypothetical protein
*Mmy*STR 3C	TGC	NED-CATTTTGGTCTCGCAGTCG	TTTTAACCACGAGCACGACA	8–20	Hypothetical protein
*Mmy*STR 3D	TGT	PET-TTCGATCACTAAGCGAAACG	GCACGGCTTTCATATCCAGT	11–27	Intergenic
*Mmy*STR 4A	TTTC	FAM-TCGTGGACGGTGCATTAAC	TCACGCGATATTTGTCAAGC	11–52	Intergenic
*Mmy*STR 4B	TGAC	VIC-CCTCGTTGTCTGAGTGAAAGC	CACGATTGGAAATGATCACA	8–29	Intergenic
*Mmy*STR 4C	AGGC	NED-CCTTGCTGAGTCCCACTGAT	GAGGGGGTTGGAGAGGAAT	5–15	Intergenic
*Mmy*STR 4D	TTCA	PET-CAGGCACCAACCAATCACTA	CAGGCACGGAGATTGAGACT	4–9	5′ UTR hypothetical protein

reaction consisted of 4 min initial denaturation at 94°C, 30 s denaturation at 94°C, 30 s annealing at 55°C, 30 s extension at 72°C, and a final extension of 7 min at 72°C.

Capillary electrophoresis. PCR products obtained were diluted 200-fold in PCR-grade water. Two microliters of diluted PCR product was combined with 0.1 μl of GeneScan 600 LIZ (Applied Biosystems) size marker and 18 μl of HiDi formamide (Applied Biosystems). The samples were denatured for 1 min at 94°C, cooled to 4°C, and injected onto an ABI 3730 XL (Applied Biosystems) genetic analyzer as recommended by the manufacturer.

Data analysis. The typing data were imported into BioNumerics software v7.6 (Applied Maths, Sint-Martens-Latem, Belgium) and analyzed using the MLVA plug-in. Assignment of repeat numbers was relative to MM55, for which the repeat numbers were taken from the genomic sequence. The genotype of MM55 is 19-17-16-14-14-13-13-17-15-10-7, which corresponds to markers 2A-2B-2C-3A-3B-3C-3D-4A-4B-4C-4D.

Comparison of typing methods. The available AFLP and VNTR data from the isolates included in this study were taken from the work of van de Sande et al. (15) and Lim et al. (16). AFLP classes and VNTR repeats used for this analysis are shown in Data Set S1. These data were then projected onto the *Mmy*STR genotypes. Furthermore, the adjusted Wallace (AW) coefficient, which is a measure of congruence between genotyping techniques, was used to compare the different typing methods to each other (28).

Genotypic diversity. To determine the genotypic diversity within the population using this panel of STR markers, Simpson's diversity index (SDI) was used. This measure is the probability that a typing assay will assign a different genotype to any two randomly chosen isolates in a microbial population for a given combination of markers. The D value has a range of 0 to 1, with 1 meaning all the isolates are different and 0 indicating that all isolates are genetically identical.

***Mmy*STR stability and specificity.** To evaluate the stability of the *Mmy*STR assay, DNA isolated from the reference strain (MM55) in 2005 and DNA extracted in 2017 after approximately 156 monthly subculturing steps were analyzed with the *Mmy*STR assay. To test for reproducibility, MM55 DNA was run four times on different days with the *Mmy*STR assay.

RESULTS

***Madurella mycetomatis* is genetically divergent.** Among the 120 *M. mycetomatis* isolates analyzed, 108 different genotypes were observed using the *Mmy*STR assay, each containing 1 to 5 isolates (Fig. 1). Of these 108 genotypes, one genotype was found in five isolates, one genotype was found in three isolates, and six genotypes were each found in two isolates. The remaining 100 genotypes were unique. The SDI for individual markers ranged from 0.081 to 0.881 (Table 2), with *Mmy*STR 2A (0.881) showing the highest level of diversity and *Mmy*STR 4C (0.081) showing the lowest diversity. When the 11 STR markers were combined, the 11-marker panel yielded a D value of 0.997.

Genotypes showed no correlation to geographical location even among the Sudanese isolates, which made up a large proportion of the tested collection, or with isolates from other geographic regions (Fig. 1). Furthermore, no correlation was observed among the Latin American isolates. Our results demonstrate that *M. mycetomatis* is genetically highly divergent.

Stability, reproducibility, and specificity of *Mmy*STR. Evaluation of the stability of the *Mmy*STR assay revealed the same number of tandem repeats for all markers following 156 instances of subculturing, confirming their temporal stability within this time

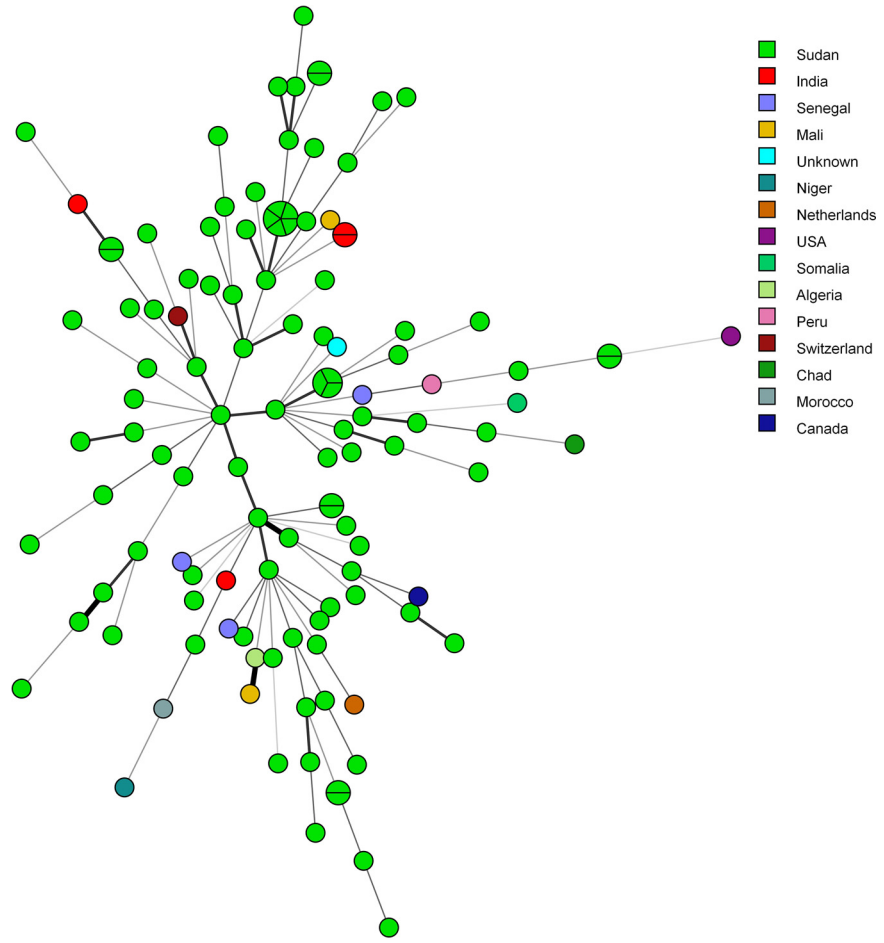

FIG 1 Minimum spanning tree based on an 11-STR-marker panel of 120 *M. mycetomatis* isolates illustrating their genetic diversity. Each circle represents a genotype; the size of each circle corresponds to the number of isolates with that genotype. Colors represent the origins of the isolates. The size and thickness of connecting lines are proportional to the number of different markers between the genotypes. One hundred eight genotypes were obtained from 120 isolates using the *Mmy*STR assay, yielding a D value of 0.997.

period. For reproducibility, running MM55 on four different occasions resulted in the same genotype, confirming the reproducibility of the assay. To test the specificity of the *Mmy*STR assay, we analyzed the following non-*M. mycetomatis* agents of eumycetoma: *M. pseudomycetomatis*, *M. tropicana*, *M. fahalii*, *F. senegalensis*, *F. tompkinsii*, and

TABLE 2 Discriminatory power (D) of individual *Mmy*STR markers and marker sets[a]

Marker set	D	Marker	D
*Mmy*STR 2	0.990	*Mmy*STR 2A	0.881
		*Mmy*STR 2B	0.754
		*Mmy*STR 2C	0.728
*Mmy*STR 3	0.980	*Mmy*STR 3A	0.631
		*Mmy*STR 3B	0.546
		*Mmy*STR 3C	0.688
		*Mmy*STR 3D	0.660
*Mmy*STR 4	0.879	*Mmy*STR 4A	0.596
		*Mmy*STR 4B	0.578
		*Mmy*STR 4C	0.081
		*Mmy*STR 4D	0.365

[a]The D value for the entire panel of markers (*Mmy*STR) was 0.997.

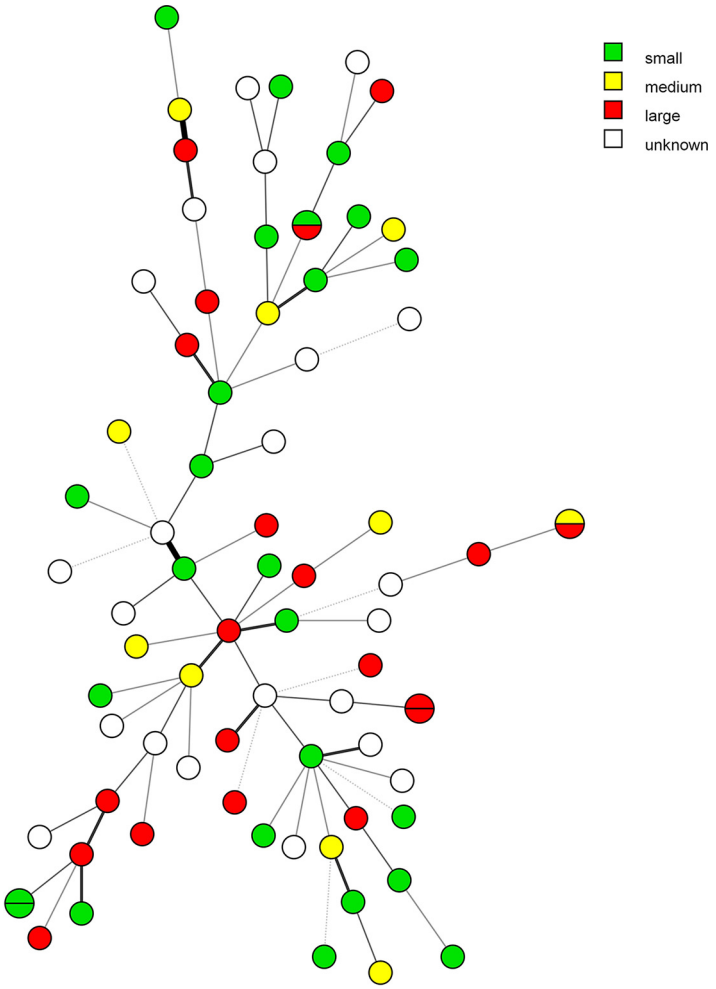

FIG 2 Minimum spanning tree of 77 isolates showing *Mmy*STR genotypes and the size of the lesion they were isolated from. Lesions were categorized as small (<5 cm), medium (5 to 10 cm), and large (>10 cm). Each circle represents a genotype; the size of each circle corresponds to the number of isolates with that genotype. Colors represent lesion sizes. The size and thickness of connecting lines are proportional to the number of different markers between the genotypes.

M. romeroi. Products were observed only with *M. tropicana* (and only for the *Mmy*STR 3C marker), showing that the panel of markers is indeed specific for *M. mycetomatis*.

***Mmy*STR genotypes show no correlation with lesion size.** In a previous study using AFLP analysis, a correlation between genotype and lesion size was demonstrated (15). We projected the lesion size for isolates for which data on the *Mmy*STR genotypes were available. The lesions were categorized as small (<5 cm), medium (5 to 10 cm), and large (>10 cm) (16). Unlike with AFLP analysis, a correlation between genotype and lesion size was not observed (Fig. 2).

The *Mmy*STR assay provides superior discrimination over AFLP and VNTR assays. Short-tandem-repeat assays are known to have superior discriminatory power compared to pattern-based techniques (19, 29). To confirm if this was also the case with the *Mmy*STR assay, we compared its data to those generated earlier using the AFLP and VNTR assays. With AFLP analysis, 33 tested isolates were grouped into 3 genotypes (1, 2, and 3) (15). When the same strains were typed using *Mmy*STR, 30 genotypes were obtained; 4 were found in 2 isolates each, while the remaining 26 were unique. The single isolate that made up class 3 (MM83) proved also to be unique using the *Mmy*STR assay (Fig. 3). Furthermore, the SDI for AFLP analysis was 0.498, while that for *Mmy*STR was 0.997 (Table 3). Using the VNTR assay, the 77 isolates tested were divided into 12 genotypes (16). When the same 77 isolates were typed with *Mmy*STR, 73

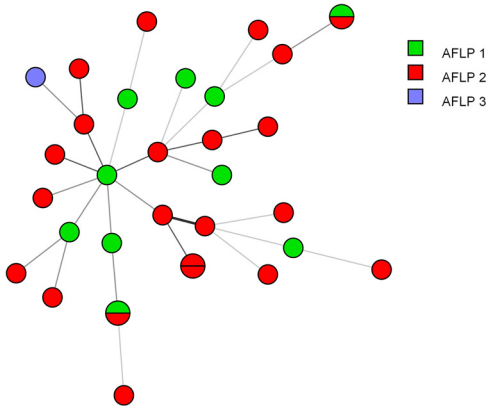

FIG 3 Minimum spanning tree showing the correlation between *Mmy*STR genotypes and AFLP genotypes. Each circle represents a genotype; the size of each circle corresponds to the number of isolates with that genotype. Colors represent AFLP genotypes. The size and thickness of connecting lines are proportional to the number of different markers between the genotypes. A collection of 33 isolates consisting of 3 AFLP genotypes resulted in 30 *Mmy*STR genotypes.

genotypes were obtained (Fig. 4), with 4 genotypes each represented by 2 isolates each and the remaining 69 genotypes being unique, resulting in an SDI of 0.767 for VNTR testing and 0.997 for *Mmy*STR (Table 3). Taken together, these data confirmed the genetic heterogeneity of *M. mycetomatis* and demonstrated the discriminatory superiority of *Mmy*STR over AFLP and VNTR testing.

Furthermore, the AW coefficients of the different typing methods were calculated. *Mmy*STR showed good congruence compared to the VNTR (0.837) and AFLP (0.713) assays (Table 4). This suggests that two isolates classified in the same *Mmy*STR genotype have an 84% probability of being classified as same genotype by VNTR and a 71% probability of being the same AFLP type. Conversely, isolates from a given AFLP genotype have only 1% and 4% probabilities of being classified in the same *Mmy*STR genotype or VNTR genotype, respectively, while the probabilities of isolates within the same VNTR genotype being classified in the same *Mmy*STR and AFLP genotype were 16% and 1%, respectively (Table 4). This further confirms that the *Mmy*STR assay is superior to AFLP analysis and the VNTR assay as a genotyping method.

DISCUSSION

The genetic variation of *M. mycetomatis* was studied previously using a variety of pattern-based techniques (12, 14–16). Among these, randomly amplified polymorphic DNA (RAPD) analysis, restriction endonuclease assay (REA), and AFLP and VNTR analyses have demonstrated different levels of genetic diversity among *M. mycetomatis* isolates (12, 15, 16), while RAPD analysis combined with restriction fragment length polymorphism (RFLP) analysis did not show any genetic diversity at all (14). Including the isolates used in previous assays, we confirmed with our *Mmy*STR assay the genetic diversity among *M. mycetomatis* isolates. Furthermore, we also demonstrated that the *Mmy*STR assay was superior to AFLP and VNTR assays for typing *M. mycetomatis*. A high level of genetic diversity has been observed in other fungal species as well using STR assays, compared to pattern-based techniques (8, 14, 17).

TABLE 3 Simpson's diversity index of three genotyping methods for *M. mycetomatis*[a]

Typing method	No. of isolates	No. of genotypes	Simpson's diversity index	CI (95%)
AFLP	33	3	0.498	0.383–0.614
VNTR	76	13	0.769	0.697–0.841
*Mmy*STR	120	108	0.997	0.966–1.000

[a]CI, confidence interval.

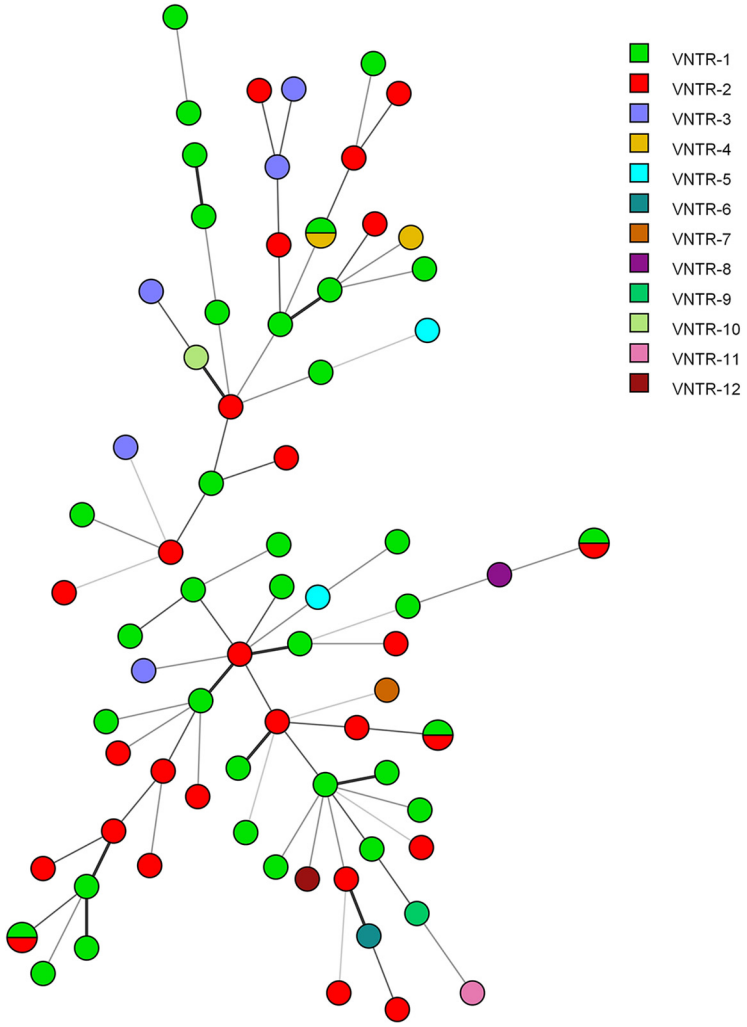

FIG 4 Minimum spanning tree showing the correlation between *Mmy*STR genotypes and VNTR genotypes. Each circle represents a genotype; the size of each circle corresponds to the number of isolates with that genotype. Colors represent VNTR genotypes. The size and thickness of connecting lines are proportional to the number of different markers between the genotypes. A collection of 77 isolates consisting of 12 VNTR genotypes resulted in 73 *Mmy*STR genotypes.

With the high discriminatory power of the *Mmy*STR assay, we did not observe any correlation with geography and lesion size. This is in contrast to the observation that isolates obtained from moderate to large lesions and originating from central Sudan were linked to AFLP cluster I. This correlation with lesion size in AFLP analysis is likely due to the presence of fragment B4, which is linked to AFLP cluster I. Fragment B4 encodes casein kinase 1δ, which is linked to DNA repair, intracellular trafficking, cell cycle progression (15), and, more importantly, virulence in certain pathogenic fungi such as *Cryptococcus neoformans* (30). In contrast, the *Mmy*STR typing technique is based on STRs found most often in noncoding regions (31), and therefore, no direct link between a

TABLE 4 Adjusted Wallace (AW) coefficients for 3 typing methods for *M. mycetomatis*

	AW coefficient (95% CI) for assay[a]		
Typing method	**AFLP**	**VNTR**	***Mmy*STR**
AFLP		0.036 (0.000–0.172)	0.010 (0.000–0.060)
VNTR	0.157 (0.000–0.536)		0.008 (0.000–0.024)
*Mmy*STR	0.713 (0.426–1.000)	0.837 (0.675–1.000)	

[a]CI, confidence interval.

certain gene and a phenotypical property is to be expected; indeed, this was also not found for other fungal infections (19, 32). When STRs are located within coding or regulatory regions, a link between phenotype and genotype can be obtained (33, 34); however, although some markers were located in coding sequences, we found no correlation to any of the studied phenotypes.

Using an STR assay, a link between genotype and geographical origin was observed in *C. neoformans* isolates obtained from various Asian countries (35). In our study, no link between genotype and geography was seen, although the majority of our isolates originated from Sudan and only 15 isolates were available from other regions in the world. Even the isolates originating from different continents clustered within the genotypes obtained for Sudan, further demonstrating the absence of a link between genotype and geography.

To establish if there is indeed a link between geographical region and genotype in *M. mycetomatis*, a larger collection of isolates from different geographical regions is needed, and we hope that scientists or physicians with *M. mycetomatis* isolates from different geographical regions are willing to collaborate with us to answer this question. In this regard, the Global Mycetoma Working Group is indispensable in establishing this link.

In conclusion, we have developed an easy, high-throughput, robust, discriminatory, and reproducible STR assay (*Mmy*STR) with an 11-marker panel to study genetic variation in *M. mycetomatis*. Furthermore, we have demonstrated that *M. mycetomatis* is genetically much more diverse than was previously shown with AFLP analysis or VNTR assay. In addition, we have demonstrated the superiority of the *Mmy*STR over the AFLP and VNTR assays. We recommend that the *Mmy*STR assay be used to establish a global database for future study of *M. mycetomatis* isolates.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, XLSX file, 0.02 MB.

REFERENCES

1. van de Sande W, Fahal A, Ahmed SA, Serrano JA, Bonifaz A, Zijlstra E, Eumycetoma Working Group. 2018. Closing the mycetoma knowledge gap. Med Mycol 56:153–164. https://doi.org/10.1093/mmy/myx061.
2. van de Sande WW. 2013. Global burden of human mycetoma: a systematic review and meta-analysis. PLoS Negl Trop Dis 7:e2550. https://doi.org/10.1371/journal.pntd.0002550.
3. van Belkum A, Fahal A, van de Sande WW. 2013. Mycetoma caused by *Madurella mycetomatis*: a completely neglected medico-social dilemma. Adv Exp Med Biol 764:179–189. https://doi.org/10.1007/978-1-4614-4726-9_15.
4. Reis CMS, Reis-Filho EGM. 2018. Mycetomas: an epidemiological, etiological, clinical, laboratory and therapeutic review. An Bras Dermatol 93:8–18. https://doi.org/10.1590/abd1806-4841.20187075.
5. Bitan O, Wiener-Well Y, Segal R, Schwartz E. 2017. Mycetoma (Madura foot) in Israel: recent cases and a systematic review of the literature. Am J Trop Med Hyg 96:1355–1361. https://doi.org/10.4269/ajtmh.16-0710.
6. Nenoff P, van de Sande WW, Fahal AH, Reinel D, Schöfer H. 2015. Eumycetoma and actinomycetoma—an update on causative agents, epidemiology, pathogenesis, diagnostics and therapy. J Eur Acad Dermatol Venereol 29:1873–1883. https://doi.org/10.1111/jdv.13008.
7. Fraser M, Borman AM, Johnson EM. 2017. Rapid and robust identification of the agents of black-grain mycetoma by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 55:2521–2528. https://doi.org/10.1128/JCM.00417-17.
8. Ahmed SA, van den Ende BH, Fahal AH, van de Sande WW, de Hoog GS. 2014. Rapid identification of black grain eumycetoma causative agents using rolling circle amplification. PLoS Negl Trop Dis 8:e3368. https://doi.org/10.1371/journal.pntd.0003368.
9. Desnos-Ollivier M, Bretagne S, Dromer F, Lortholary O, Dannaoui E. 2006. Molecular identification of black-grain mycetoma agents. J Clin Microbiol 44:3517–3523. https://doi.org/10.1128/JCM.00862-06.
10. Segretain G, Mariat F. 1968. Research on the presence of agents of mycetoma in the soil and thorny plants of Senegal and Mauritania. Bull Soc Pathol Exot Filiales 61:194–202.
11. Zijlstra EE, van de Sande WWJ, Welsh O, Mahgoub ES, Goodfellow M, Fahal AH. 2016. Mycetoma: a unique neglected tropical disease. Lancet Infect Dis 16:100–112. https://doi.org/10.1016/S1473-3099(15)00359-X.
12. Lopes MM, Freitas G, Boiron P. 2000. Potential utility of random amplified polymorphic DNA (RAPD) and restriction endonuclease assay (REA) as typing systems for *Madurella mycetomatis*. Curr Microbiol 40:1–5. https://doi.org/10.1007/s002849910001.
13. van de Sande WW, Fahal AH, Goodfellow M, Mahgoub eS, Welsh O, Zijlstra EE. 2014. Merits and pitfalls of currently used diagnostic tools in mycetoma. PLoS Negl Trop Dis 8:e2918. https://doi.org/10.1371/journal.pntd.0002918.
14. Ahmed A, van de Sande W, Verbrugh H, Fahal A, van Belkum A. 2003. *Madurella mycetomatis* strains from mycetoma lesions in Sudanese patients are clonal. J Clin Microbiol 41:4537–4541. https://doi.org/10.1128/JCM.41.10.4537-4541.2003.
15. van de Sande WW, Gorkink R, Simons G, Ott A, Ahmed AO, Verbrugh H, van Belkum A. 2005. Genotyping of *Madurella mycetomatis* by selective amplification of restriction fragments (amplified fragment length polymorphism) and subtype correlation with geographical origin and lesion size. J Clin Microbiol 43:4349–4356. https://doi.org/10.1128/JCM.43.9.4349-4356.2005.
16. Lim W, Eadie K, Horst-Kreft D, Ahmed SA, Fahal AH, van de Sande WWJ.

2018. VNTR confirms the heterogeneity of Madurella mycetomatis and is a promising typing tool for this mycetoma causing agent. Med Mycol 57:434–440. https://doi.org/10.1093/mmy/myy055.
17. Klaassen CH. 2009. MLST versus microsatellites for typing *Aspergillus fumigatus* isolates. Med Mycol 47:S27–33. https://doi.org/10.1080/13693780802382244.
18. de Valk HA, Meis JF, Curfs IM, Muehlethaler K, Mouton JW, Klaassen CH. 2005. Use of a novel panel of nine short tandem repeats for exact and high-resolution fingerprinting of *Aspergillus fumigatus* isolates. J Clin Microbiol 43:4112–4120. https://doi.org/10.1128/JCM.43.8.4112-4120.2005.
19. Rudramurthy SM, de Valk HA, Chakrabarti A, Meis JF, Klaassen CH. 2011. High resolution genotyping of clinical *Aspergillus flavus* isolates from India using microsatellites. PLoS One 6:e16086. https://doi.org/10.1371/journal.pone.0016086.
20. Gits-Muselli M, Peraldi MN, de Castro N, Delcey V, Menotti J, Guigue N, Hamane S, Raffoux E, Bergeron A, Valade S, Molina JM, Bretagne S, Alanio A. 2015. New short tandem repeat-based molecular typing method for *Pneumocystis jirovecii* reveals intrahospital transmission between patients from different wards. PLoS One 10:e0125763. https://doi.org/10.1371/journal.pone.0125763.
21. Gillis T, Vissa V, Matsuoka M, Young S, Richardus JH, Truman R, Hall B, Brennan P, Partners IC, Ideal Consortium Partners. 2009. Characterisation of short tandem repeats for genotyping *Mycobacterium leprae*. Lepr Rev 80:250–260. https://doi.org/10.47276/lr.80.3.250.
22. Illnait-Zaragozi MT, Martínez-Machín GF, Fernández-Andreu CM, Boekhout T, Meis JF, Klaassen CH. 2010. Microsatellite typing of clinical and environmental *Cryptococcus neoformans* var. grubii isolates from Cuba shows multiple genetic lineages. PLoS One 5:e9124. https://doi.org/10.1371/journal.pone.0009124.
23. de Groot T, Puts Y, Berrio I, Chowdhary A, Meis JF. 2020. Development of Candida auris short tandem repeat typing and its application to a global collection of isolates. mBio 11:e02971-19. https://doi.org/10.1128/mBio.02971-19.
24. de Valk HA, Meis JF, Bretagne S, Costa JM, Lasker BA, Balajee SA, Pasqualotto AC, Anderson MJ, Alcázar-Fuoli L, Mellado E, Klaassen CH. 2009. Interlaboratory reproducibility of a microsatellite-based typing assay for *Aspergillus fumigatus* through the use of allelic ladders: proof of concept. Clin Microbiol Infect 15:180–187. https://doi.org/10.1111/j.1469-0691.2008.02656.x.
25. Gupta V, Dorsey G, Hubbard AE, Rosenthal PJ, Greenhouse B. 2010. Gel versus capillary electrophoresis genotyping for categorizing treatment outcomes in two anti-malarial trials in Uganda. Malar J 9:19. https://doi.org/10.1186/1475-2875-9-19.
26. Benson G. 1999. Tandem repeats finder: a program to analyze DNA sequences. Nucleic Acids Res 27:573–580. https://doi.org/10.1093/nar/27.2.573.
27. Smit S, Derks MF, Bervoets S, Fahal A, van Leeuwen W, van Belkum A, van de Sande WW. 2016. Genome sequence of *Madurella mycetomatis* mm55, isolated from a human mycetoma case in Sudan. Genome Announc 4:e00418-16. https://doi.org/10.1128/genomeA.00418-16.
28. Severiano A, Pinto FR, Ramirez M, Carriço JA. 2011. Adjusted Wallace coefficient as a measure of congruence between typing methods. J Clin Microbiol 49:3997–4000. https://doi.org/10.1128/JCM.00624-11.
29. de Valk HA, Meis JF, de Pauw BE, Donnelly PJ, Klaassen CH. 2007. Comparison of two highly discriminatory molecular fingerprinting assays for analysis of multiple *Aspergillus fumigatus* isolates from patients with invasive aspergillosis. J Clin Microbiol 45:1415–1419. https://doi.org/10.1128/JCM.02423-06.
30. Wang Y, Liu TB, Patel S, Jiang L, Xue C. 2011. The casein kinase I protein Cck1 regulates multiple signaling pathways and is essential for cell integrity and fungal virulence in *Cryptococcus neoformans*. Eukaryot Cell 10:1455–1464. https://doi.org/10.1128/EC.05207-11.
31. Fan H, Chu JY. 2007. A brief review of short tandem repeat mutation. Genomics Proteomics Bioinformatics 5:7–14. https://doi.org/10.1016/S1672-0229(07)60009-6.
32. Hadrich I, Neji S, Drira I, Trabelsi H, Mahfoud N, Ranque S, Makni F, Ayadi A. 2013. Microsatellite typing of *Aspergillus flavus* in patients with various clinical presentations of aspergillosis. Med Mycol 51:586–591. https://doi.org/10.3109/13693786.2012.761359.
33. Winter R, Liebold J, Schwarz E. 2013. The unresolved puzzle why alanine extensions cause disease. Biol Chem 394:951–963. https://doi.org/10.1515/hsz-2013-0112.
34. Alanio A, Desnos-Ollivier M, Garcia-Hermoso D, Bretagne S. 2017. Investigating clinical issues by genotyping of medically important fungi: why and how? Clin Microbiol Rev 30:671–707. https://doi.org/10.1128/CMR.00043-16.
35. Pan W, Khayhan K, Hagen F, Wahyuningsih R, Chakrabarti A, Chowdhary A, Ikeda R, Taj-Aldeen SJ, Khan Z, Imran D, Sjam R, Sriburee P, Liao W, Chaicumpar K, Ingviya N, Mouton JW, Curfs-Breuker I, Boekhout T, Meis JF, Klaassen CH. 2012. Resistance of Asian *Cryptococcus neoformans* serotype A is confined to few microsatellite genotypes. PLoS One 7:e32868. https://doi.org/10.1371/journal.pone.0032868.